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Commentary

Nerve Growth Factor: The Dark Side of the Icon

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Nerve growth factor (NGF), the archetypal neurotrophic polypeptide, has been extensively characterized for its involvement in the development and maintenance of neurons in the peripheral and central nervous system. The awarding of the Nobel prize in 1986 to Rita Levi-Montalcini for the discovery of NGF and the subsequent demonstration of the existence of a family of related neurotrophic factors, the neurotrophins, have placed NGF in the position of an iconic emblem. From the stimulation of neuron survival and differentiation and the guidance of nerve fibers to the sculpting of organ innervation, the concepts first established with NGF have proven to be universally true for all other neurotrophic factors. Thus, it is now clear that the development of the nervous system and the somatic innervation are driven by the stimulatory action of neurotrophic molecules. However, even from early days, there were hints this icon could have a "dark side," that NGF might be involved in pathogenesis. For example, NGF was reported early on to be produced by some cancer cells[1] or to be mitogenic for both lymphocytes[2] and keratinocytes.[3] Although these findings could have been the impetus for further investigations into related pathologies, they were regarded as anecdotal and were clearly overshadowed by other exciting developments in the field. The amino acid sequencing of NGF made it the first growth factor to be fully sequenced,[4] other NGF-related peptides were evidenced, and there was also the discovery of the two NGF receptors, the death receptor $p75^{NTR}$ and the tyrosine kinase receptor TrkA. Subsequently, elucidation of NGF-associated signaling was performed in neuronal or neuron-like cells such as the pheochromocytoma PC12 cells, which differentiate to extend neurites under NGF stimulation. Nevertheless, time has passed and ideas about NGF are now increasingly revised to include its involvement in the initiation and progression of pathologies. The dark side of the icon is progressively unveiled, and NGF appears to be intimately involved in several major pathologies characterized by deregulation of cell survival, proliferation, and differentiation, such as in cancer or during the inflammatory process. In this issue of *The American Journal of Pathology*, Raychaudhuri et al[5] provide a remarkable illustration of the pathological involvement of NGF in the case of psoriasis. Most notably, this work reveals that the impact of NGF in psoriasis is related not only to its effects on keratinocyte proliferation and inflammation but also to its neurotrophic activity, emphasizing the role of the nervous system in this pathology.

Psoriasis is a common skin disease characterized by a thickening of the epidermis due to increased proliferation and altered differentiation of keratinocytes, resulting in characteristic plaques over the skin.[6] In addition to epidermal hyperplasia, major histological features include a pro-inflammatory reaction with infiltrating leukocytes and dilated blood vessels in the dermis. It is worth noting that the processes of keratinocyte proliferation, reactionary inflammation, and angiogenesis are all known to be affected by NGF. Keratinocytes have long been described to produce NGF, with a resulting autocrine loop able to stimulate their proliferation.[3,7,8] NGF has also been shown to activate T lymphocytes, to induce the release of inflammatory mediators,[9] and to trigger angiogenesis through endothelial cell multiplication.[10] Thus, although the basis for NGF intervention in psoriasis has been known, the beauty of the work presented here by Raychaudhuri et al[5] is to have clarified a rational means of NGF intervention. This was elegantly achieved by taking advantage of the Koebner phenomenon, which describes the appearance of psoriatic lesions in the uninvolved skin of patients as a consequence of a trauma. The action of creating damage in the skin by tape stripping thus results in the appearance of psoriatic lesions and can be regarded as a model for studying the disease. First, the authors observed that cultures of keratinocytes from the nonlesional skin of psoriatic patients produced 10 times more NGF than keratinocytes from healthy individuals. Then, a clear up-regulation of NGF in Koebner lesions was observed starting as early as 24 hours after trauma, with a peak in the second week. This therefore suggests that NGF overexpression by keratinocytes is an early event in the development of psoriasis.

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This is an important conclusion because keratinocytes have sometimes been presented as simple bystanders in psoriasis that secondarily react to other stimulating agents. Here, by demonstrating that cells from the nonlesional skin of patients overexpress NGF, the study not only points to this trophic factor as a key player but also replaces keratinocytes at the center of the pathogenic cascade of psoriasis.

In the second part of the work, psoriatic plaques were xenografted into SCID-immunodeficient mice, with normal skin serving as control. The intriguing observation here was that the psoriatic plaques induced the sprouting of nerve fibers into such transplants, further demonstrating that the NGF produced by keratinocytes is indeed functionally active and that it can induce neuritogenesis. Although neuritogenesis is not currently regarded as a major event in psoriasis, there are indications that it might play a significant role. For example, sprouting of nerve fibers can be observed in psoriatic plaques as well as the up-regulation of neuropeptides such as substance P.[11,12] The results reported by Raychaudhuri et al[5] reveal that the increase in the number of nerve fibers entering psoriatic plaques specifically concerns the $p75^{NTR}$-positive nerve fibers. $P75^{NTR}$ is part of the bipartite NGF receptor system with TrkA, and this result demonstrates that those nerve fibers entering the plaques are directly sensitive to NGF, with no need to invoke an indirect, intermediate mechanism involving other factors. It should be noted that neuritogenesis is a difficult phenomenon to study *in situ*, particularly in the epidermis. These NGF-reactive sensory nerve fibers, which are usually unmyelinated, are difficult to reveal as they have very thin diameters—typically about 1/10 the size of a keratinocyte. Therefore, the study of neuritogenesis in skin is challenging, probably accounting, at least partially, for the fact that we have much less information on the role of neuritogenesis compared with angiogenesis in psoriasis pathology.

The fact that psoriasis, as well as many other skin diseases, is related to or exacerbated by stress[13] has long provided a basis for the idea that the nervous system is involved, and the present work of Raychaudhuri et al[5] points to a mechanism involving the invasion of skin lesions by nerve fibers themselves. Although the mechanism by which NGF-induced nerve fibers could actually participate in the pathogenesis of psoriasis is yet to be determined, several hypotheses suggest themselves. It is known that outgrowing nerve fibers have a trophic effect during embryogenesis and regenerative processes, notably by producing and secreting a large array of mitogenic growth factors. Whether or not the nerve fibers growing into psoriatic plaques also release growth factors requires further investigation, but Raychaudhuri et al[5] demonstrate here that nerve fiber sprouting is associated with increased levels of the neuropeptide substance P. Interestingly, the neuropeptides that are known to be released in psoriasis also participate in the inflammation characteristic of the disease.[14]

The molecular pathways leading to inflammation in psoriasis are multiple and involve many cytokines such as interferon, tumor necrosis factor, and nitric oxide as well as several proteases.[15] The initiating event in inflammatory psoriasis is considered to be T lymphocyte invasion, but the amplification of the process also involves a vast range of cytokine-mediated activities. NGF itself is clearly not an innocent bystander with regard to immunity and inflammation. It is an autocrine survival factor for memory B lymphocytes,[16] and it can activate T lymphocytes and induce the release of other inflammatory mediators.[9] Mast cells also synthesize, store, and release NGF,[17] and, importantly, neurogenic inflammation can be promoted by NGF.[18] Taken together, the work of Raychaudhuri et al[5] provides a concrete underpinning of the idea that the skin, the nervous system, and the immune system do not operate independently but rather are closely associated with and exploit the common language of cytokines—NGF clearly being one of them.

The idea of targeting NGF receptors to alleviate psoriasis has been tentatively explored by use of a local application of the TrkA pharmacological inhibitor K252a, which appears to result in a significant improvement in the clinical as well as histological features of the condition.[19] However, K252a cannot be regarded as a particularly specific inhibitor of TrkA as it also exhibits a wide range of inhibition toward several other tyrosine kinase receptors. Importantly, because receptor tyrosine kinases are virtually ubiquitous over a wide variety of cell types, local application of K252a leads to tissue necrosis, so it is unlikely that this could ever be used in human therapy. However, more targeted approaches can be envisioned, such as the use of NGF-blocking antibodies that would specifically block NGF, thus narrowing the effect to keratinocytes, nerve fibers, and immunity, without affecting other tyrosine kinase receptors. As NGF production is localized in keratinocytes, the targeted use of siRNA against NGF, although prospective at this stage, can be imagined. Nevertheless, further preclinical investigations are obviously required to assess the potential value of NGF as a therapeutic target in psoriasis.

In a broader context, NGF has been increasingly described in another type of disease characterized by a deregulation of cell growth and differentiation as well as inflammation: cancer. Within the nervous system, neuroblastoma cell growth can be inhibited with NGF; it is therefore not a promoter of brain cancer development in this etiology. However, in carcinomas, ie, cancers originating from epithelial cells, the involvement of NGF and its receptors in the promotion of tumor development has been described, as is notably the case in prostate,[20] breast,[21] and pancreatic[22] cancers. Similarly to what is reported by Raychaudhuri et al[5] for psoriasis, in breast cancer, NGF is overexpressed, acts through an autocrine loop involving TrkA and $p75^{NTR}$, and is regarded as a potential therapeutic target[23–25]; in pancreas, NGF stimulates both pancreatic cell survival and innervation,[26] a process that may also occur during prostate carcinogenesis.[27] This homology between NGF production and biological activities in both psoriasis and cancer points to common aspects in the two pathologies, with NGF as a common player.

More than 50 years after the discovery of NGF as a neurotrophic factor essential for nervous system devel-

opment, the other side of the icon, the deregulation of cell growth and differentiation in various pathologies, provides a rationale for exploring the potential value of NGF and its receptors as both markers and therapeutic targets. To date, NGF-related strategies for diagnostic, prognostic, and therapeutic application have been explored mainly in the field of neuroscience, where the goal has generally been to enhance its neurotrophic activity or compensate for its down-regulation. An extension of such concepts is now desirable, with the design of anti-NGF strategies as well as their translation into practical applications constituting major challenges for the future.

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